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(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Immunological Precipitation Process for the Determination of a Bindable Analyte, as Well as a Reagent for Carrying Out This Process

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(30) (DE) P 40 34 509.2 1990/10/30

(57) 26 Claims

Canada

ABSTRACT

Immunological precipitation process for the determination of a bindable analyte, as well as a reagent for carrying out this process

The present invention provides an immunological precipitation process for the determination of a bindable analyte by incubation of a sample solution which contains the analyte with a specific receptor bindable with the analyte, wherein to the test solution is added a non-ionic polymer from the group comprising dextran with a molecular weight of more than 500,000, polyvinylpyrrolidone with a molecular weight of more than 100,000 and polyethylene glycol with a molecular weight of more than 10,000, reagent for carrying out the immunological precipitation process is also provided which in addition to the materials necessary for the immunological precipitation process, contains the non-ionic polymer.

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The present invention is concerned with an immunological precipitation process for the determination of a bindable analyte by incubation of a sample solution, which contains the analyte, with a specific receptor bindable with the analyte, as well as a reagent suitable therefor.

Immunological precipitation processes have long been used for the determination of bindable analytes. Use is thereby made of the fact that many immunological reactions lead to molecule aggregates or, in the case of an agglutination reaction, to particle aggregates, which differ considerably in the diffused light behaviour from the starting materials and the concentration of which can be determined by way of this property. For the quantitative determination of analytes, the light scattering on particles, which are present in a homogeneous medium, is utilised not only via the measurement of the diffused light intensity (nephelometry) but also via the measurement of the loss of intensity of the light beam passing through the medium (turbidimetry).

A fundamental problem of all quantitative immunological precipitation processes is given from the shape of the reaction curve. If, in the case of constant antibody concentration, increasing amounts of antigen are added, then a typical course of the curve is given for the precipitation (Heidelberger curve).

In the zone of the antibody excess, the concentration of the precipitate and thus the measurement signal increases. In the case of further addition of antigen, the curve passes through a maximum and again decreases in the zone of the antigen excess. On the basis of this effect, a measurement signal can be associated to two antigen concentration values.

In the case of immunological methods of determination, this effect is also referred to as the "high dose hook effect", hereinafter briefly referred to as the "hook effect". In the case of many analytes and especially in the case of proteins, the occurring highest possible physiological concentration lies far beyond the maximum of the Heidelberger curve so that these possibilities of error occur very frequently. In order to avoid these errors, in the case of the determination it must be ascertained whether the measurement signal is present in the ascending or descending limb of the Heidelberger curve.

The oldest and most certain method was described by H.E. Schulze and G. Schwick, Prot. Biol. Fluids, 5, 15-25/1958, which provides for a double determination

with two different sample dilutions. In the case of the antigen excess, with the more highly diluted sample, there is measured a greater measurement signal than with the more concentrated sample.

An improved embodiment of this process provides for a further addition of antibodies. In the case of the presence of an excess of antigen, a signal increase occurs (T.O. Tiffany et al., Clin. Chem., 20, 1005-1061/1974). By additional pipetting of antigen material of known concentration, an antigen excess can also be recognised (J.C. Sternberg, Clin. Chem., 23, 1456-1464/1977).

Furthermore, several processes have been described which seek to recognise the hook effect by laborious computor-controlled evaluations. By determination of the period of reaction up to the appearance of the maximum reaction velocity in the case of nephelometric measurements, it is possible to discriminate between antigen and antibody excess (DE-A-27 24 722; EP-B-0,148,463).

These processes all suffer from the disadvantage that either an additional pipetting step or a computor-controlled evaluation is necessary and, in the case of automation, this automatically leads to an increase of the cost of the apparatus. A better possibility of avoiding erroneous interpretations in the case of immunological precipitation processes would be so to

change the form of the Heidelberger curve that, after the maximum, a plateau is reached and the appearance of the hook effect is completely avoided or is displaced into an antigen concentration range which no longer occurs under physiological conditions.

In U.S. Patent Specification No. 4,595,661 are described sandwich and nephelometric immunoassays which, for the reduction of the hook effect, besides the highly specific antibodies and reagents normally present in an immunoassay, additionally contain at least one further low-affine antibody against the analyte. The disadvantage of this process is obvious since, for each analyte to be detected, there must also be prepared two specific antibodies with different affinities for the analyte.

Therefore, the present invention seeks to provide a simple immunological precipitation process for the determination of a bindable analyte which avoids the hook effect as disturbance.

Thus, according to the present invention, there is provided an immunological precipitation process for the determination of a bindable analyte by incubation of a sample solution which contains the analyte with a specific receptor bindable with the analyte, wherein to the test solution is added a non-ionic polymer from the group comprising dextran with a molecular weight of more than 500,000, polyvinylpyrrolidone with a

molecular weight of more than 100,000 and polyethylene glycol with a molecular weight of more than 10,000.

The present invention also provides a reagent for carrying out an immunological precipitation process for the determination of a bindable analyte in a sample solution, wherein, in addition to the materials necessary for the immunological precipitation process, it contains a non-ionic polymer from the group comprising dextran with a molecular weight of more than 500,000, polyvinylpyrrolidone with a molecular weight of more than 100,000 and polyethylene glycol with a molecular weight of more than 10,000.

The non-ionic polymer concentration in the test batch is at least 1% by weight. Below this concentration, the action according to the present invention is no longer achieved. The upper amount is given by the appearance of non-specific turbidities in the case of high polymer concentrations. In the test batch, a non-ionic polymer concentration of 2 to 6% by weight has proved to be advantageous, a concentration of from 3 to 4% by weight being especially preferred. The reagent according to the present invention can be present as powder. lyophilisate or solution.

As non-ionic polymers,.there can be used, for example, polyethylene glycol, polyvinyl pyrrolidone or dextran. Non-ionic polymers are obtainable in differing degrees of polymerisation, i.e. with different

molecular weights. For the present invention, high molecular weight non-ionic polymers are preferred, the upper limit of the molecular weight thereby depending upon the molecular weight at which the polymer is no longer sufficiently soluble in the test batch in order to be effective according to the present invention. For use in the process according to the present invention, polyethylene glycol has a molecular weight of more than 10,000, preferably a molecular weight of from 10,000 and 300,000 and quite especially a molecular weight of 40,000. Polyvinylpyrrolidone has a molecular weight of more than 100,000 and preferably of 360,000 to 750,000. Dextran is preferably used with a molecular weight of more than 200,000 and especially preferably with a molecular weight of from 500,000 to 1,000,000.

It was known, by means of the addition of polymers, such as polyethylene glycol, dextran or hyaluronic acid, in the case of immunological precipitation processes, to increase the sensitivity and to add them as reaction accelerators. Polyethylene glycol with a molecular weight of about 6000 is usually employed in a concentration of approximately 4% by weight in the case of nephelometric or turbidimetric determinations of an immunological precipitation reaction (EP-B-0,148,463).

It was surprising that the addition according to the present invention of non-ionic polymers with the said molecular weights and concentrations avoids, in the case of immunological precipitation processes, the hook effect as disturbance which occurs in the case of high antigen concentrations, i.e. the hook effect is avoided or can be displaced into an antigen concentration range which no longer occurs under physiological conditions. At the same time, by means of this addition, the sensitivity and velocity of the immunological precipitation reaction is increased and often beyond the amount which is achieved by the otherwise usual addition of polyethylene glycol 6000. Thus, the addition of polyethylene glycol 6000 is no longer necessary in the process according to the present invention. In the case of the process according to the present invention, a false antigen concentration can no longer be measured since, in the case of increasing antigen concentration, the measurement signal, after passing the maximum of the precipitation curve, no longer decreases so strongly or only decreases in the case of non-physiologically high antigen concentrations that it again comes to lie in the measurement range. A discrimination between the ascending and descending limb of the Heidelberger curve, as was previously necessary by means of an additional pipetting step or of a computor-controlled evaluation in the case of the

processes of the prior art, is thus unnecessary. The process according to the present invention can be carried out without increased costs in comparison with the previously usual processes since the use of the non-ionic polymers according to the present invention can replace the polyethylene glycol 6000 previously usually added. In addition, in the case of automated use, the cost of apparatus can be reduced since additional pipetting steps or a laborious evaluation can be omitted.

By an immunological precipitation process in the meaning of the present invention is to be understood all reactions between immunological receptors and analytes which lead to a turbidity of the test solution as the result of the formation of the analyte-receptor complex. The turbidity can thereby be strengthened by light-scattering particles to which one component of the immunological reaction, i.e. receptor or analyte or analyte analogue, is or can be bound. In this case, it is an agglutination test. The non-ionic polymer according to the present invention could be used especially preferably in such immunological precipitation processes wherein the turbidity is caused only by analyte receptor complexes and which processes do not contain light-scattering particles. Furthermore, by immunological precipitation processes are also to be understood immune diffusion processes and preferably the radial immune diffusion.

In the scope of the present invention, the analyte and receptor can, in principle, be all substance pairings which are bindable with one another. In this definition, in the scope of the present invention

apart from immunological bindable substance pairs, there are also included substances pairings which behave analogously.

As bindable, specific receptors, in the meaning of the present invention are to be understood binding components of the analyte to be analysed. As receptor, there are preferably used antibodies or antibody fragments. In the case of antibodies or antibody fragments, these can be not only polyclonal but also monoclonal antibodies.

By an analyte is to be understood a substance which has at least two epitopes, i.e. binding positions for the specific receptor. The process according to the present invention is especially preferred when the analyte is a protein. The analyte can be present in a body fluid, for example plasma, serum, urine, saliva or the like, or in an appropriate buffer solution. The process according to the present invention is, for example, especially suitable for the determination of albumin in urine, for the determination of apolipoproteins AI and B in serum or plasma and for the determination of immunoglobulins, ferritin, Lp(a) and α-1-microglobulin. In the case in which the analyte itself is an antibody, as specific receptor there can be used the antigen reacting with this antibody or, however, an anti-antibody directed against this antibody.

The measurement of the turbidity in the case of the immunological precipitation process according to the present invention can take place with appropriate apparatus not only nephelometrically but also turbidimetrically. The determination of the concentration of the analyte in the sample takes place by comparison with a standard of known analyte concentration.

By a reagent for carrying out the immunological precipitation process for the determination of a bindable analyte in a sample solution, there is to be understood a composition which, in addition to the materials necessary for the immunological precipitation process, for example adjuvants, buffer substances or, in the case of an agglutination test, particles coated with a binding component, preferably latex particles, also contains a non-ionic polymer which is effective according to the present invention. The concentration of this non-ionic polymer in the reagent is thereby to be so chosen that, in the test batch, i.e. after addition of the sample to the reagent, the end concentration of the polymer is at least 1 and preferably 2 to 6% by weight.

The invention is further explained and illustrated by reference to the accompanying drawings in which:

FIG. 1 illustrates graphically the Heidelberger curve in a reaction curve in a reaction curve of a quantitative immunological precipitation process, at constant antibody concentration; and

FIGS. 2 to 8 illustrate graphically avoidance of the hook effect as a disturbance, in accordance with the invention.

With further reference to Fig. 1, there is shown the Heidelberger curve, for the case of constant antibody concentration. As explained previously in the zone of the antibody excess, the concentration of the precipitate and thus the measurement signal increases. In the case of further addition of antigen, the curve passes through a maximum and again decreases in the zone of the antigen excess. On the basis of this effect, a measurement signal can be associated to two antigen concentration values.

If, in the case of the antigen excess (C_2), the measurement signal lies within the measurement range (C_0 - C_M) for which the ascending limb of the Heidelberger curve is suitable, then the false antigen concentration F_1 is read off.

As explained previously, this is the "hook effect".

The follow Examples are given for the purpose of illustrating the present invention:

Example 1.

Determination of human albumin in urine.

In urine diagnosis, the determination of albumin is an important criterion of the assessment of kidney damage. The normal value of albumin in urine is from 10 to 20 mg/l but physiological concentrations of up to 20,000 mg/l are possible. For the avoidance of erroneous interpretations, the hook effect should be completely avoided in the case of albumin determinations or displaced into the concentration range above 20,000 mg/l. As a rule, the normal measurement range in the case of turbidometric determinations only extends up to an albumin concentration of 300 mg/l.

The carrying out of the experiment, as well as the reagents employed, are the same in the following Examples with the exception of the addition of the non-ionic polymer for the avoidance of the hook effect as disturbance:

Solution 1 (reaction buffer):

50 mmol/l Tris, pH 8.0

1% by wt. non-ionic detergent

0.1% by wt. sodium azide

To solution 1 were added the non-ionic polymers or, as comparison with the process of the prior art, PEG 6000 in concentrations such that, in the test batch, there is achieved the particular given end concentration.

Solution 2 (antiserum):

100 mmol/l Tris, pH 7.2

100 mmol/l sodium chloride

0.1% by wt. sodium azide

15 mg/ml polyclonal anti-human serum albumin sheep antibody (PAB<HSA>S-IgG)

Solution 3 (calibrator):

50 mmol/l phosphate buffer, pH 8.0

100 mmol/l sodium chloride

0.1% by wt. sodium azide

0 to 20,000 mg/l human albumin (HSA).

The measurements were carried out bichromatically at a wavelength of 340 nm (correction wavelength 700 nm) on a Hitachi 704* of Boehringer Mannheim GmbH, Germany, at a temperature of 37°C. 20 µl of solution 3 were mixed with 350 µl of solution 1 and incubated for 5 minutes. Thereafter, the first measurement of the extinction took place (E1). 70 µl of solution 2 were pipetted thereto and the test batch incubated for a further 5 minutes. Thereafter, a further extinction measurement (E2) took place. For the evaluation of the results, the extinction difference $\Delta E = E2 - E1$ was plotted against the albumin concentration.

1.1. Avoidance of the hook effect by the addition of polyethylene glycol.

1.1.1. Comparison between the process of the prior art (addition of PEG 6000) and the process according to the present invention (addition of PEG 40,000).

* Trade Mark

To solution 1 was added PEG 6000 or PEG 40,000 in such concentrations that, in each case, an end concentration of 4% by weight was achieved in the test batch. The test was carried out as described under 1.

In the case of the process of the prior art, i.e. the addition of PEG 6000, the hook effect took place in the case of albumin concentrations above the measurement range which extended to about 400 mg/l HSA. In the case of the addition according to the present invention of PEG 40,000, with HSA concentrations above the measurement range, no significant decrease of the extinction resulted. Up to an HSA concentration of about 20,000 mg/l, which is possible under physiological conditions, the hook effect is avoided as disturbance (see Fig. 2 of the accompanying drawings). Furthermore, it was shown that the process according to the present invention led to an acceleration of the reaction and to an increase of the sensitivity of the immunological precipitation process which extended beyond the measure that is achieved by the process of the prior art.

1.1.2. Influence of the concentration of PEG 40,000.

PEG 40,000 was added to solution 1 in different concentrations. The end concentration in the test batch was from 0 to 6% by weight. The use of still higher concentrations is no longer of meaning since above 6% by weight of PEG 40,000, non-specific turbidities occur which prevent an exact measurement.

In the case of PEG 40,000 concentrations of up to 1% by weight, the hook effect occurred above HSA concentrations of 400 mg/l (see Fig. 3 of the accompanying drawings). Above 1% by weight of PEG 40,000, there was obtained a distinct decrease of the hook effect in comparison with the method of the prior art (4% by weight PEG 6000, the values are not graphically shown in Fig. 3). From these results, it can be seen that the non-ionic polymer according to the present invention can be used in concentrations of at least 1 and preferably of 2 to 6% by weight and especially preferably of 3 to 4% by weight.

1.1.3. Influence of the molecular weight of polyethylene glycol.

In order to determine the influence of the molecular weight of PEG on the immunological precipitation, the polymer was used with different degrees of polymerisation. PEG with a molecular weight of 2000, 6000, 10,000, 40,000 and 300,000 were tested. The particular concentration in the test batch was uniformly 4% by weight.

Above the molecular weight of 10,000, the hook effect was avoided as disturbance. Especially preferably, there is used PEG with a molecular weight of 40,000 (see Fig. 4 of the accompanying drawings).

1.2. Determination of albumin in urine with the addition of dextran.

As in the case of Example 1.1.3, the influence of the molecular weight of a further non-ionic polymer, in this case dextran, in the process according to the present invention is determined using the example of the determination of albumin in urine. In each case, the concentration of the dextrans in the test batch was 4% by weight.

Above a molecular weight of 500,000, dextran can be used as additive for the avoidance of the hook effect as disturbance in the case of immunological precipitation processes. Dextrans with a lower molecular weight do not achieve the action according to the present invention (see Fig. 5 of the accompanying drawings).

1.3. Determination of albumin in urine with PVP addition.

Using the example of albumin determination, there is determined the influence of the molecular weight of polyvinyl pyrrolidone (PVP) on the process according to the present invention. PVP was, in each case, used in an end concentration of 4% by weight, referred to the test batch.

Above a molecular weight of more than 100,000, PVP can be used for the avoidance of the hook effect as disturbance. As can be seen from Fig. 6 of the accompanying drawings, in the case of PVP with a molecular weight of 360,000 and 750,000, no significant

lowering of the extinction took place above the measurement range whereas PVP with a molecular weight of 10,000 does not achieve this effect.

Example 2.

Determination of apolipoprotein A-I (Apo A-I).

In the case of the determination of the concentration of apolipoprotein A-I, the experiments were carried out as described in Example 1. The measurements were carried out bichromatically at a wavelength of 376 nm (correction wavelength 700 nm) on a Hitachi 704 of Boehringer Mannheim GmbH, Germany, at a temperature of 30°C.

The following reagent solutions were used:

Solution 1 (reaction buffer):

50 mmol/l Tris, pH 8.0

1% by weight non-ionic detergent

To the solution was added PEG 40,000 or, as comparison with the process of the prior art, PEG 6000 in such concentrations that an end concentration of 4% by weight was achieved in the test batch.

Solution 2 (antiserum):

There was used the antiserum obtainable from Boehringer Mannheim GmbH under the identification No. 1381130 which contains the polyclonal anti-apolipoprotein A1 sheep antibody (PAB <Apo A1> S-IgG) in 100 mmol Tris, pH 7.2.

Solution 3 (calibrator):

As calibrator, there was used lyophilised human serum which is obtainable from Boehringer Mannheim GmbH under the identification No. 1381156.

2 µl of solution 3 were mixed with 350 µl of solution 1 and incubated for 5 minutes. Thereafter, the first extinction measurement took place (E1). 140 µl of solution 2 were pipetted thereto, the test batch was incubated for a further 5 minutes and a second extinction measurement (E2) was carried out. For the evaluation, the extinction difference ΔE = E2 - E1 was plotted against the apolipoprotein A-I concentration.

The calibration curve was measured analogously to the package insert (identification No. 1378686) of the Tina-quant® apolipoprotein A-I test kit of Boehringer Mannheim GmbH. The calibrator, which contained 211 mg of apolipoprotein A-I/dl, was thereby diluted with a 0.9% by weight sodium chloride solution in different dilution steps. The high apolipoprotein A-I concentrations were achieved by the pipetting of the undiluted calibrator with comparatively high volumes (2 µl. 4 µl etc.).

From Fig. 7 of the accompanying drawings, it can be seen that, in the case of the process according to the prior art, i.e. in the case of the use of PEG 6000, a hook effect occurs above an apo-A-I concentration

of about 210 mg. By means of the use according to the present invention of PEG 40,000, the hook effect is displaced into a higher concentration range.

Example 3.

Determination of α-1-microglobulin (α-1-M).

The measurements were carried out on a Hitachi 704 as in the preceding Examples. The measurement temperature was 37°C. Measurement was carried out at a wavelength of 340 nm with a correction wavelength of 700 nm.

The following reagent solutions were used:

Solution 1 (reaction buffer):

50 mmol/l Tris, pH 8.0

150 mmol/l sodium chloride

1% by weight non-ionic detergent, i.e. PEG 6000 and PEG 40,000, in such concentrations that an end concentration of 4% by weight was achieved in the test batch.

Solution 2 (antiserum):

20 mg/l of polyclonal anti-α-1-microglobulin sheep IgG antibody (PAB <α-1-M> S-IgG) in 50 mmol/l Tris, pH 8.0.

Solution 3 (calibrator):

As calibrator, there was used a urine concentrate with an α-1-microglobulin content of 2700 mg/l.

20 µl of solution 3 were mixed with 350 µl of solution 1 and, after 5 minutes, the extinction (E1) was measured. After the addition of 70 µl of

solution 2 and incubation for a further 5 minutes, a second extinction (E2) was measured. For the evaluation, the extinction difference $\Delta E = E2 - E1$ was plotted (Fig. 8 of the accompanying drawings). For the production of a calibration line, the calibrator was diluted in 20 mmol/l of hepes.

In the case of the use of PEG 6000 (process according to the prior art), a hook effect was observed above an α-1-M concentration of 1000 g/l. In the case of the use of PEG 40,000, over the whole of the measurement range up to a concentration of 2700 mg/l, no hook effect was observed.

The patent specifications referred to herein are more fully identified hereinafter:

European Patent Specification 0,148,463, E. Metzmann et al, published (laid open) October 5, 1988, assigned to Behringwerke AG.

U.S. Patent 4,595,661, L.K. Cragle et al, issued June 17, 1986, assigned to Beckman Instruments, Inc.

German Offenlegungsschrift (laid open specification) 27 24 722, R. Anderson et al, published (laid open) December 8, 1977, assigned to Beckman Instruments, Inc.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. An immunological precipitation process for the determination of a bindable analyte by incubation of a test batch of a sample solution which contains the analyte with a specific receptor bindable with the analyte, wherein to the test portion is added a non-ionic polymer from the group consisting of dextran with a molecular weight of more than 500,000, polyvinylpyrrolidone with a molecular weight of more than 100,000 and polyethylene glycol with a molecular weight of more than 10,000.

2. A process according to claim 1, wherein said non-ionic polymer is added to the test batch in a concentration of at least 1% by weight.

3. A process according to claim 2, wherein the non-ionic polymer is present in the test batch in a concentration of 2 to 6% by weight.

4. A process according to claim 1, 2 or 3, wherein said non-ionic polymer is polyethylene glycol with a molecular weight of from 10,000 to 300,000.

5. A process according to claim 4, wherein said non-ionic polymer is polyethylene glycol with a molecular weight of 40,000.

6. A process according to claim 1, 2 or 3, wherein said non-ionic polymer is polyvinylpyrrolidone with a molecular weight of from 360,000 to 750,000.

7. A process according to claim 1, 2, 3 or 5, wherein it is an agglutination test.

8. A process according to claim 1, 2, 3 or 5, wherein the specific receptor is a polyclonal antibody.

9. A process according to claim 1, 2, 3 or 5, wherein the specific receptor is a mixture of at least two monoclonal antibodies.

10. A process according to claim 1, 2, 3 or 5, wherein the analyte is a protein.

11. A process according to claim 1, 2, 3 or 5, wherein the analyte is albumin, apolipoprotein A-I or α-1-microglobulin.

12. A process according to claim 4, wherein the analyte is albumin, apolipoprotein A-I or α-1-microglobulin.

13. A praocess according to claim 6, wherein the analyte is albumin, apolipoprotein A-I or α-1-microglobulin.

14. A process according to claim 4, wherein the specific receptor is a polyclonal antibody.

15. A process according to claim 4, wherein the specific receptor is a mixture of at least two monoclonal antibodies.

16. A process according to claim 6, wherein the specific receptor is a polyclonal antibody.

17. A process according to claim 6, wherein the specific receptor is a mixture of at least two monoclonal antibodies.

18. A process according to claim 14, 15, 16 or 17, wherein the analyte is albumin, apolipoprotein A-I or α-1-microglobulin.

19. A reagent for carrying out an immunological precipitation process for the determination of a bindable analyte in a sample solution, comprising materials for the immunological precipitation process, and, additionally, a non-ionic polymer from the group consisting of dextran with a molecular weight of more than 500,000, polyvinylpyrrolidone with a molecular weight of more than 100,000 and polyethylene glycol with a molecular weight of more than 10,000.

20. A reagent according to claim 19, wherein the non-ionic polymer concentration in a test batch of the sample solution is at least 1% by weight.

21. A reagent according to claim 20, wherein the non-ionic polymer concentration in the test batch is 2 to 6% by weight.

22. The use of a non-ionic polymer for the avoidance of the hook effect as disturbance in immunological precipitation processes.

23. The use of a non-ionic polymer as reaction accelerator, for the increase of the sensitivity and for the avoidance of the hook effect as disturbance in immunological precipitation processes.

24. Use of polyethylene glycol with a molecular weight of more than 10,000 for the avoidance of the hook effect as a disturbance in an immunological precipitation process.

25. Use of polyvinylpyrrolidone with a molecular weight of more than 100,000, for the avoidance of the hook effect as a disturbance in an immunological precipitation process.

26. Use of dextran with a molecular weight of more than 500,000, for the avoidance of the hook effect as a disturbance in an immunological precipitation process.

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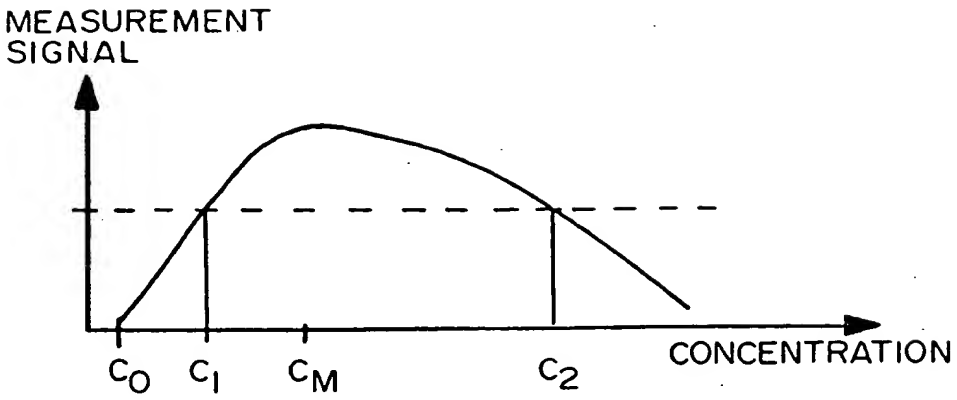

Fig.1

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Swabey Ogilvy Renault

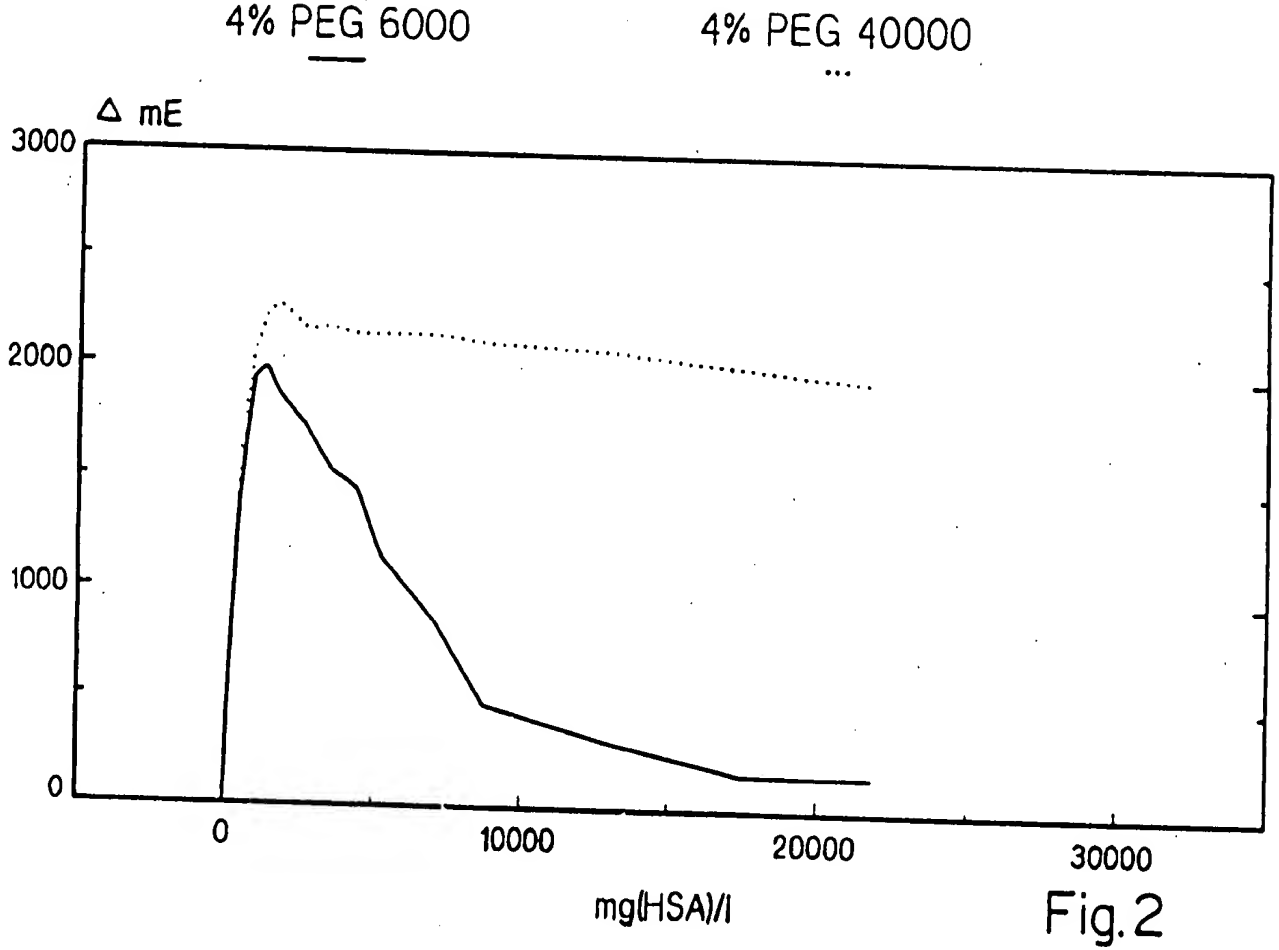

Fig. 2

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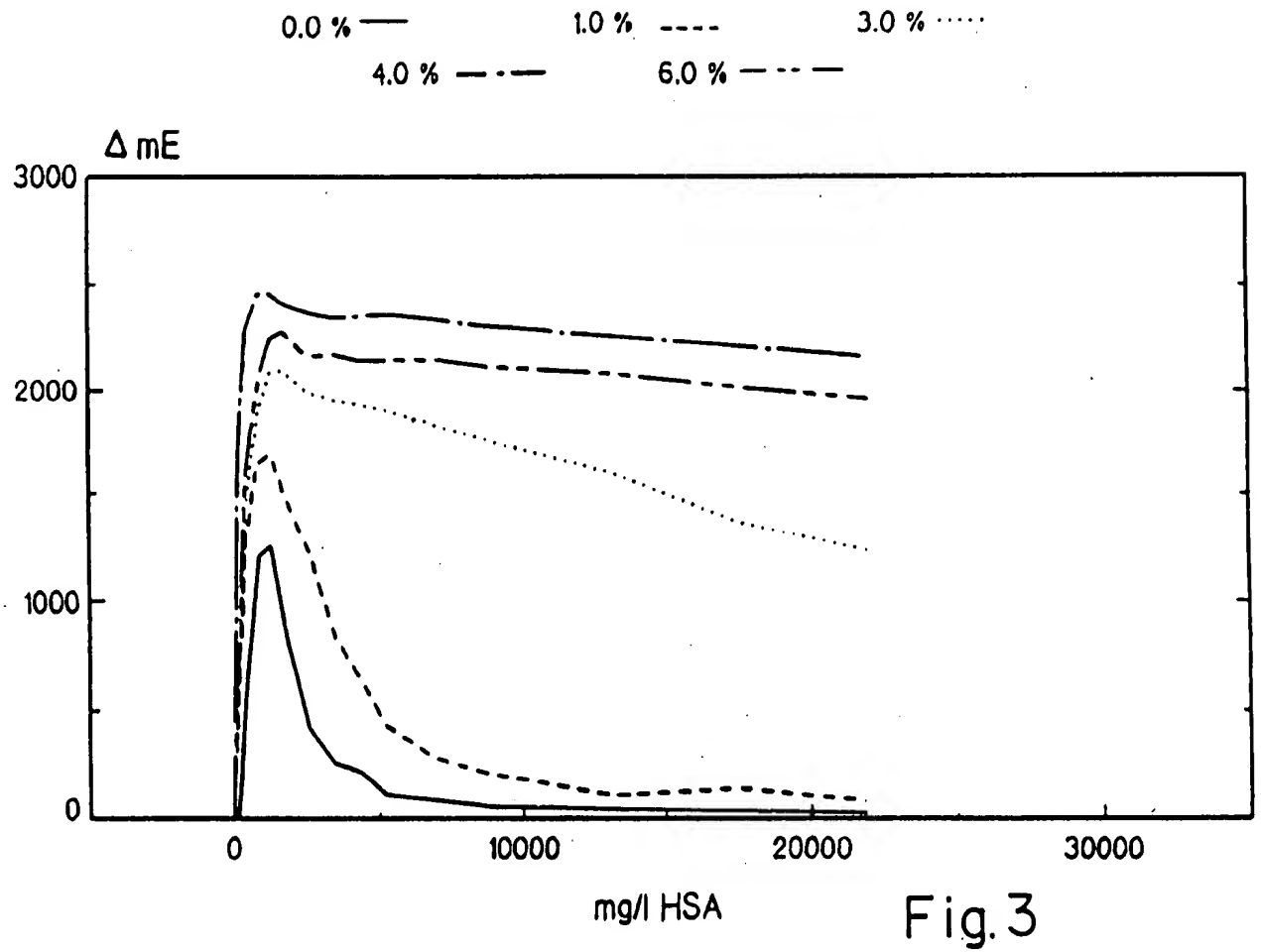

Fig. 3

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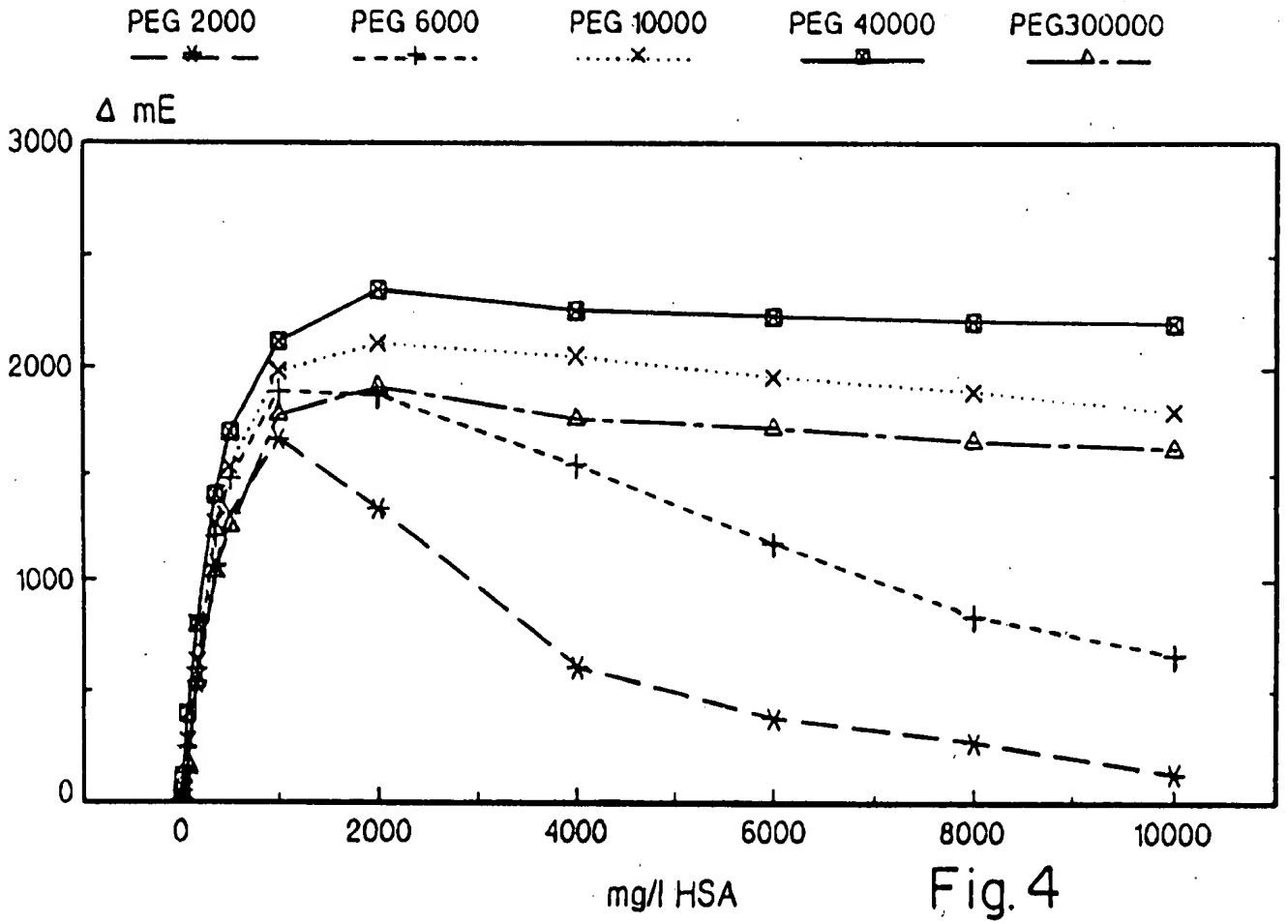

Fig. 4

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Swabey Ogilvy Renault

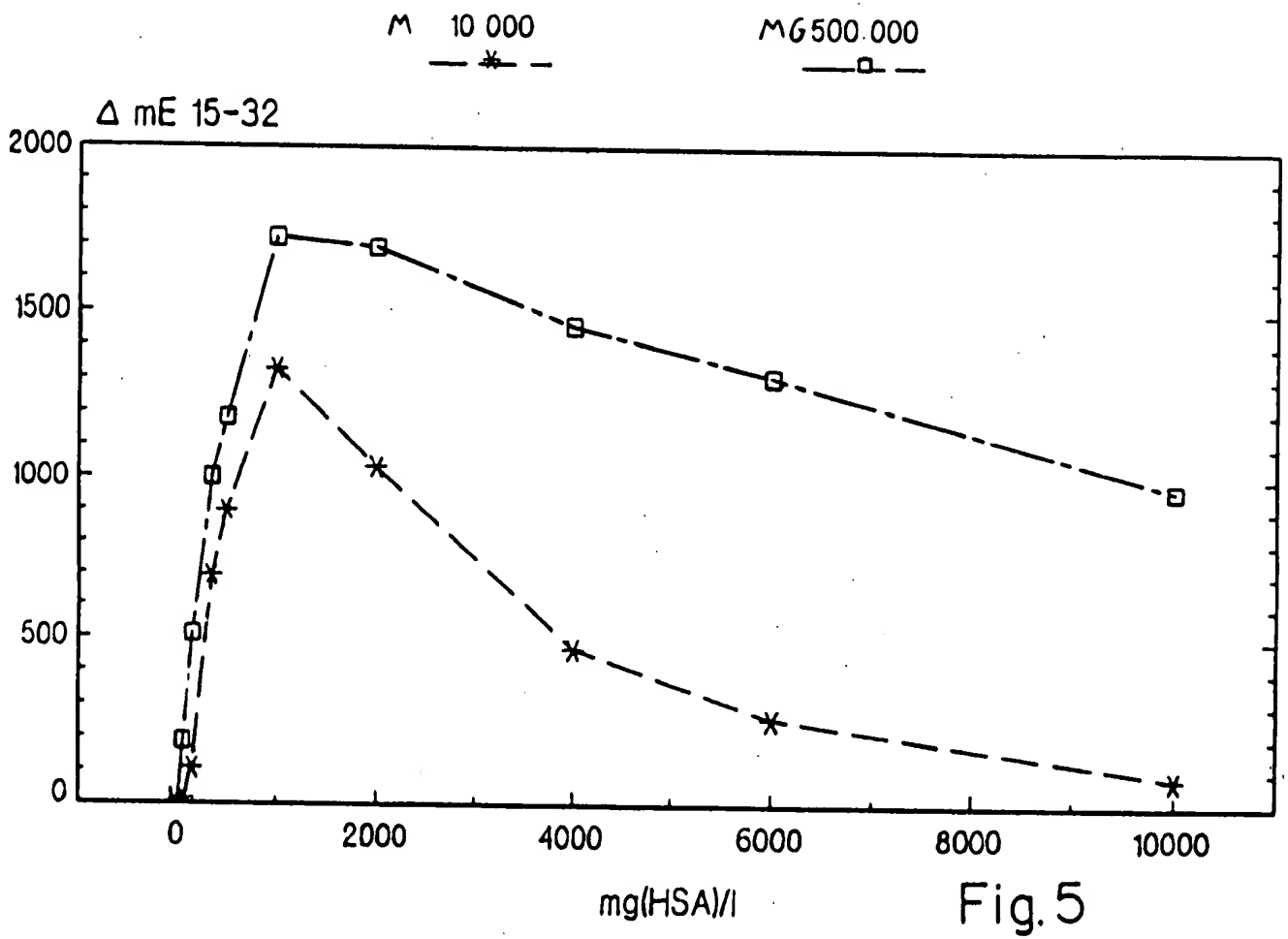

Fig. 5

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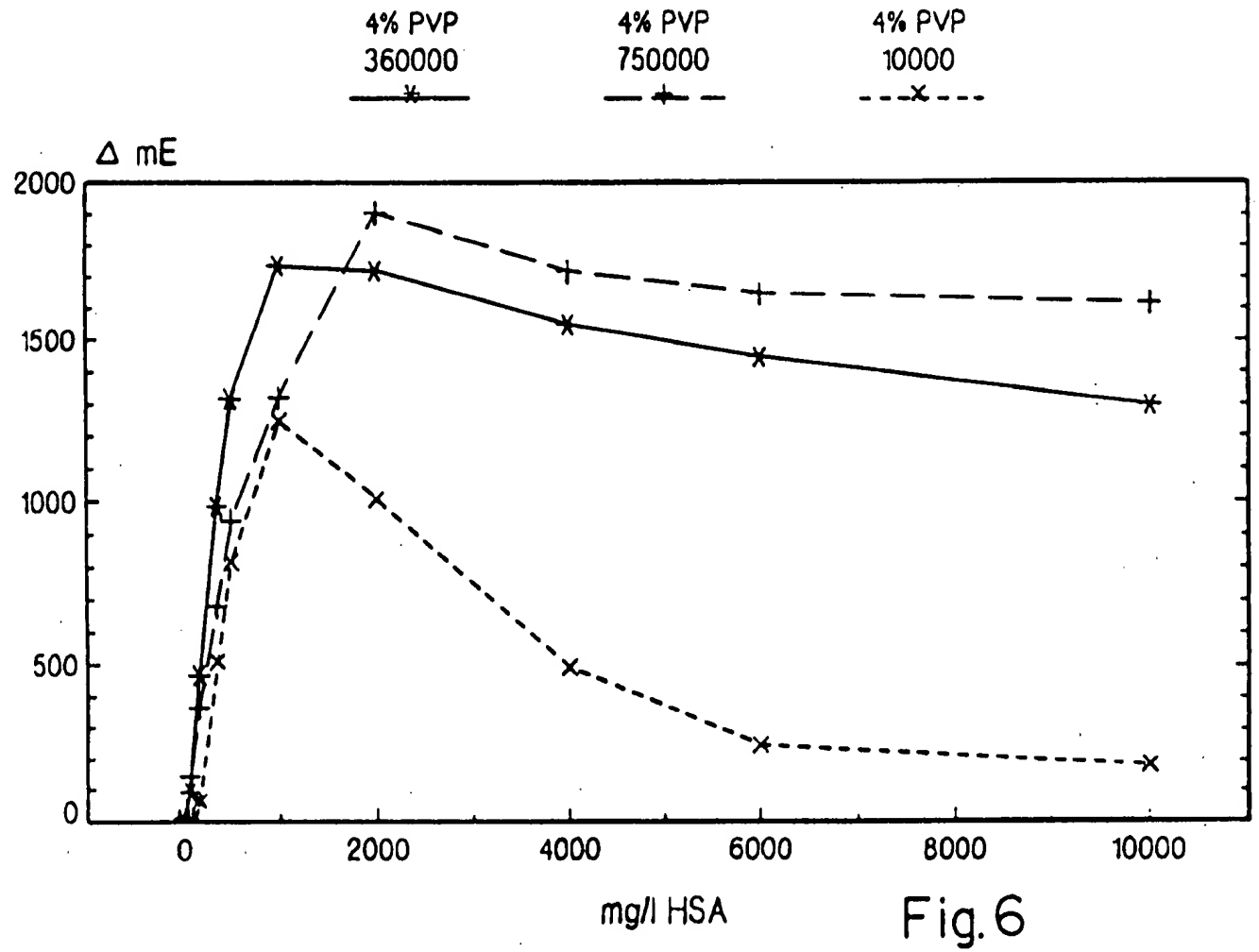

Fig. 6

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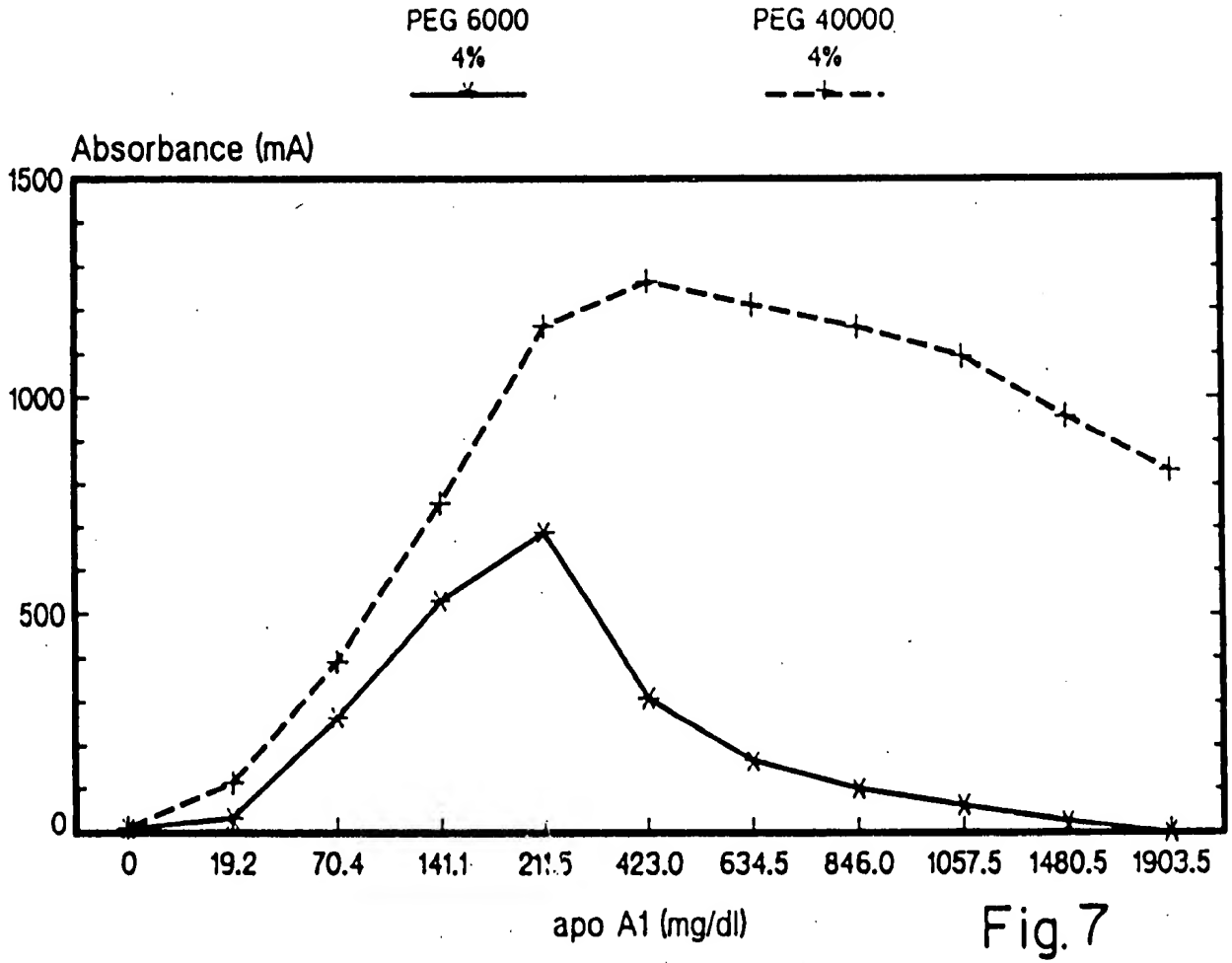

Fig. 7

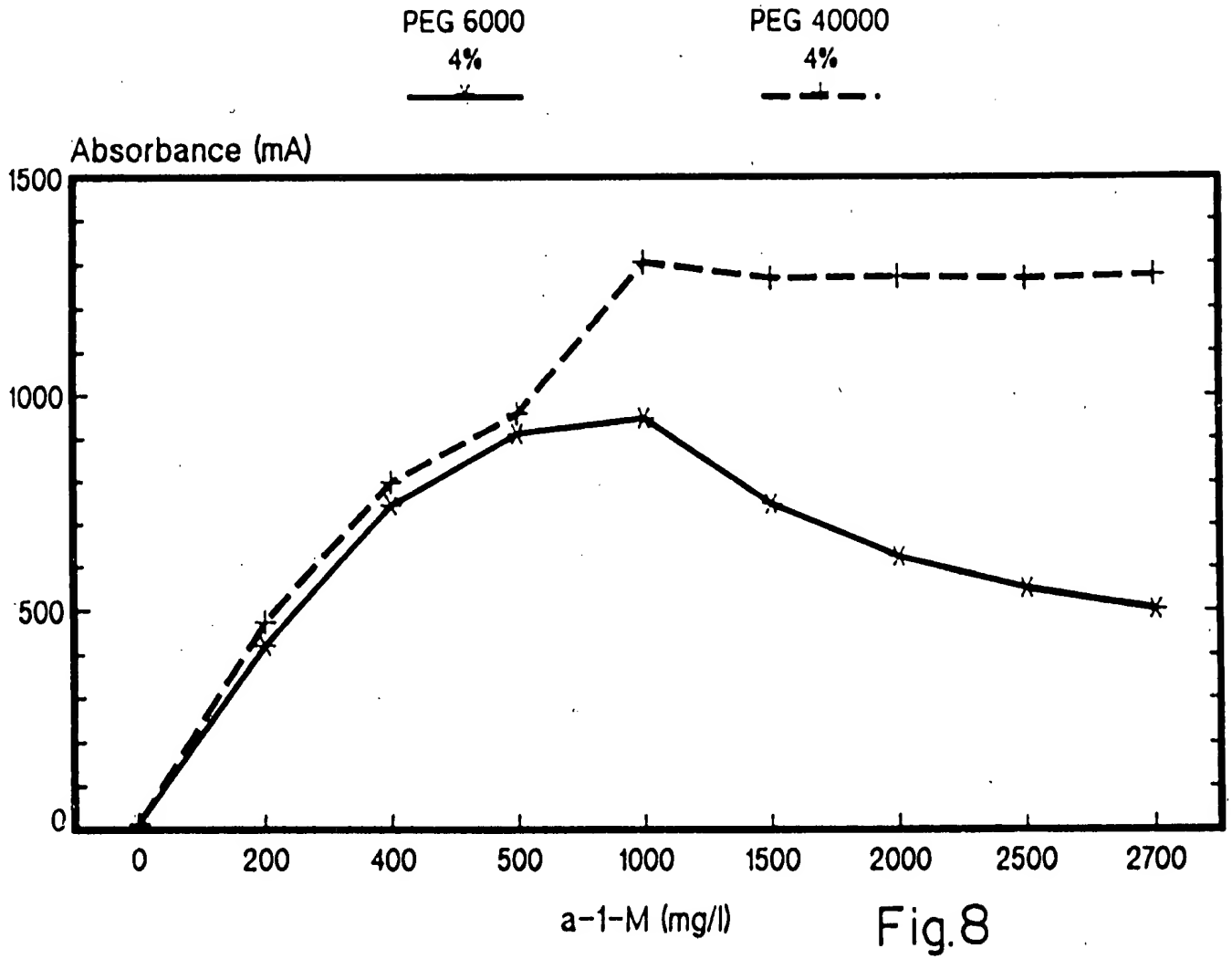

Fig.8

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Swabey Ogilvy Renault